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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of
Inventor(s): PILARSKI, Linda

Appln. No.: 09/142,557
Series code ↑ ↑ serial no.
Filing Date: September 11, 1998

Title: METHODS FOR CELL MOBILIZATION USING IN VIVO TREATMENT WITH HYALURONAN

Group Art Unit: 1623

Examiner: K. Fonda

Atty. Dkt: PM 098810 / 0300893
Client # / Matter #

Certificate of Mailing

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By: Karyn F. Massie

* * * * * * *

CERTIFICATE UNDER 37 C.F.R. § 3.73(b)

On behalf of the Governors of the University of Alberta, the undersigned certifies that the University of Alberta is the assignee of the entire right, title and interest in the patent application identified above by virtue of a chain of title from the inventors of the patent application identified above as shown below:

1. On March 5, 1997, the inventor assigned all rights and interest in the invention and in Canadian Patent Application No. 2,173,272 and any patent application based on or derived from this application to Hyal Pharmaceutical Corporation (copy enclosed). The above-identified application is the U.S. National phase of PCT/CA97/00172, which claims priority to Canadian Patent Application No. 2,173,272. Thus, the assignment from the inventor to Hyal Pharmaceutical Corporation from Canadian Patent Application No. 2,173,272 also applies to the instant application.

2. From PricewaterhouseCoopers, Inc. (as receiver for Hyal Pharmaceutical Corporation) to SkyePharma, PLC., recorded February 16, 2001 at Reel 011497, Frame 0288.

3. From SkyePharma, PLC., to Jagotec AG, recorded February 16, 2001 at Reel 011497, Frame 0879.

4. From Jagotec AG, to the Governors of the University of Alberta, recorded August 30, 2001 at Reel 012118, Frame 0552.

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Pilarski, L. -
Application No. 09/142,557

The undersigned has reviewed all the documents in the chain of title of the present application and, to the best of the undersigned's knowledge and belief, title is in the assignee identified above.

The undersigned is empowered to sign this certificate on behalf of the assignee.

The undersigned declares further that all statements made herein on personal knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are made punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code on that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: JANUARY 14, 2003 By:

Name:
Title:
On behalf of:
THE GOVERNORS OF THE
UNIVERSITY OF ALBERTA

LAINE WOOLLARD, B.Sc. (Pharm), LL.B
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PCT/CA 97/00172

18 MARCH 1997 (18.03.97)

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RECD 25 MAR 1997
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La présente atteste que les documents ci-joints, dont la liste figure ci-dessous, sont des copies authentiques des documents déposés au Bureau des brevets.

This is to certify that the documents attached hereto and identified below are true copies of the documents on file in the Patent Office.

Specification and Drawings, as originally filed, with Application for Patent Serial No: 2,173,272, on April 2, 1996, by HYAL PHARMACEUTICAL CORPORATION, assignee of Linda May Pilarski, for "Stimulation of Stem Cells and Other Cells".

PRIORITY DOCUMENT

S. Gregoire

Agent certificateur/Certifying Officer

March 18, 1997

Date

Industrie Canada Industry Canada

(CIPO 68)

Canada

OPIC
Office de la propriété intellectuelle du Canada

CIPO
Canadian Intellectual Property Office

Ottawa Hull K1A 0C9

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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Stimulation of Stem Cells and Other Cells

(72) Pilarski, Linda May - Canada ;

(71) HYAL PHARMACEUTICAL CORPORATION - Canada ;

(57) 43 Claims

Notice: This application is as filed and may therefore contain an incomplete specification.

Industrie Canada Industry Canada

OPIC - CIPO 191

Canada

ABSTRACT

The use of forms of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof is provided for the same purposes known for recombinant GM-CSF and includes the use of stimulating stem cells.

TITLE OF INVENTION

Stimulation of the Production/Release of Stem Cells and Other Cells

FIELD OF THE INVENTION

This invention relates to the use of forms of hyaluronan for stimulation of increased stem cell production/release from the bone marrow into the blood in a human and also in a number of aspects, to the stimulation of progenitor cell production/release, precursor cell production/release, accessory cell production/release, and white cell production/release into the blood in a human.

BACKGROUND OF INVENTION

The cells that populate the blood are all derived from multipotential (or pluripotential) stem cells present in bone marrow. Multipotential stem cells continually proliferate and renew themselves, but also give rise to common progenitor cells. Once committed, progenitor cells differentiate into immature precursor cells of the various blood cell lineages which, following further differentiation stages, eventually give rise to mature functional blood cells, such as erythrocytes, monocytes, lymphocytes, and polymorphonuclear cells. Terminally differentiated blood cells generally lose their ability to proliferate - indeed mammalian erythrocytes and platelets contain no nuclei - and thus have finite lifetimes. Granulocytes exist only for a matter of hours, whereas human erythrocytes remain in circulation for over 100 days and some lymphocytes have life-spans measured in years. Therefore, to maintain steady-state numbers of particular blood cell types, there must be a continual production of these from the bone marrow. This process is known as haemopoiesis (haematopoiesis) or

haemopoietic process. While much remains to be learned, it is clear that many steps in the haemopoietic process (haemopoiesis) are controlled by certain cytokines, also known as haemopoietic growth factors.

A number of cytokines now fall into the category of haemopoietic growth factors. Some of the haemopoietic growth factors appear to be very pleiotropic, i.e. act on many cell types, while others appear to be restricted to particular blood cell lineages. IL-1, IL-3, IL-6, and GM-CSF (Granulocyte-Macrophage Colony Stimulating Factor) could be considered to form one group of haemopoietic growth factors which is 'promiscuous' regarding cell type, whereas G-CSF, M-CSF, IL-5, and erythropoietin (EPO) form another group of factors with limited target-cell specificity.

The mature cells of the haemopoietic system are erythrocytes, granulocytes, lymphocytes, monocytes, macrophages, mast cells, and platelets. These all have a limited life-span, and must be replaced as they die. To achieve a balance between cell death and renewal, the bone marrow must not only continuously provide progenitor cells, but also control the commitment of these to the various lineages so that the correct proportions of mature cells are produced. The basic control mechanisms, especially of the earliest stages of haemopoiesis, are as yet poorly understood. There appears to be some compartmentalization of the marrow, and microscopic 'nests' of particular precursor cells have been identified. However, it has been shown that the survival and proliferation of stem and progenitor cells is dependent upon the presence of accessory cells which *in vitro* form into an adherent 'stromal' layer. In the absence of the stromal layer, stem and progenitor cells die and so it appears the stromal cells support proliferation and differentiation by intercellular interactions including production of growth factors into the extracellular milieu. In culture, stromal cells have been shown to produce GM-CSF, M-CSF, and a

megakaryocyte-colony stimulating factor (or molecules functionally equivalent to these). It is widely believed that such growth factors (cytokines) are involved in haemopoiesis, but their exact role(s) in self-renewal of stem cells, differentiation of stem cells into common progenitor cells, and the proliferation and differentiation of committed progenitor cells, remains unclear. More definite roles of these cytokines in the growth stimulation and development of later-stage precursors have been evinced by the use of *in vitro* colony-forming culture systems introduced by Metcalf and colleagues in the 1970s. In these experimental systems multipotential stem cells, progenitors, or precursors are suspended in the absence of stromal cells in semi-solid agar growth medium. Without the addition of exogenous cytokines, the cells die. However, they can be stimulated to grow, multiply, and differentiate to form colonies of various blood cell lineages by adding into the growth medium dilutions of certain supernatants obtained from activated leukocytes or by addition of the now readily available purified recombinant cytokines including GM-CSF. Furthermore, injection of recombinant cytokines into experimental animals, and into patients in clinical trials to assess therapeutic potential of individual cytokine products, has shown that IL-3, GM-CSF, and G-CSF stimulate the production of white cells such as granulocytes and monocytes, thus lending support for physiological roles of such cytokines. In addition, it has also become apparent that these cytokines not only support the growth and differentiation of immature blood cells, but also in many instances are effector molecules for the functional activation of mature cells.

The molecular cloning of both murine and human homologues of IL-3, GM-CSF, G-CSF, M-CSF, IL-5, and EPO has been accomplished.

Of the four 'granulocyte-macrophage' CSFs, GM-CSF was the first to be isolated and characterized. GM-CSF was shown to induce the proliferation of murine bone marrow - or spleen-derived haemopoietic cells containing

granulocyte and macrophage progenitors giving rise to colonies containing mainly granulocyte and macrophage precursors. In this respect, GM-CSF appears to share biological properties with the subsequently characterized IL-3. However, more recent studies suggest that GM-CSF acts on 'later-stage' multipotential cells than IL-3. Also, GM-CSF appears to be less active than IL-3 in stimulating the proliferation of erythroid and megakaryocytic precursors. Nevertheless, like IL-3, GM-CSF can be shown to have activities in mature cells of the granulocyte and macrophage lineages.

GM-CSF (Granulocyte-Macrophage Colony Stimulating Factor) acts directly and selectively on granulocyte/macrophage progenitors to stimulate growth and differentiation *in vitro* of cells belonging to these lineages, e.g. neutrophils, eosinophils, macrophages. These pleiotropic activities have also been demonstrated for recombinant GM-CSF. Besides regulation of the proliferation and differentiation of the progenitor/precursor cells of the myeloid lineage, GM-CSF has also been shown to activate the functions of mature myeloid cell types. For example, GM-CSF has been found to induce macrophage tumoricidal activity against the malignant melanoma cell line, A375. IFNg can also behave as a macrophage activating factor, but in contrast to GM-CSF requires an additional secondary stimulus, e.g. bacterial LPS, to evoke tumoricidal activity. In addition, GM-CSF activates macrophages to inhibit the replication of *Trypanosoma cruzi* (a unicellular parasite that is the aetiological agent of Chagas disease, or American trypanosomiasis) and increases respiratory oxidative processes. Furthermore, the replication of HIV-1 in the human monocytic cell line U937 has been shown to be moderately inhibited by GM-CSF, and more effectively by the combination of GM-CSF and IFNg. These results suggest that GM-CSF could have a potential physiological role in eosinophils and macrophage activation and thus possibly could be used prophylactically or therapeutically against a range of microbial agents that replicate in macrophages.

In neutrophils and eosinophils, GM-CSF stimulates a number of functions. In particular, GM-CSF enhances phagocytosis of bacteria and yeasts by neutrophils. Purified recombinant human GM-CSF has also been shown to enhance the cytotoxic activity of neutrophils and eosinophils against anti-body-coated target cells. These observations and others in which the anti-microbial functions of neutrophils and eosinophils are increased by GM-CSF, strongly suggest an important role for this mediator in host defence.

When mice are repeatedly injected intraperitoneally with recombinant murine GM-CSF, there is a rapid and sustained increase in the number and functional activity of peritoneal macrophages, granulocytes (neutrophils and eosinophils) as well as increased numbers of circulating monocytes. (GM-CSF usually takes about two weeks to act.) Marked increases in neutrophil, eosinophil, and monocyte numbers have also been observed following injection of recombinant human GM-CSF into AIDS patients and non-human primates. However, there may be complications associated with GM-CSF therapy. Metcalf and colleagues have shown that transgenic mice containing a constitutively expressed murine GM-CSF gene have pathological lesions soon after birth in various tissues, including lens, retina, and striated muscle, resulting from activated-macrophage infiltration. Thus, chronic macrophage activation in GM-CSF therapeutic schedules should be avoided. (Activated macrophages are known to produce a number of inflammatory mediators including cytokines such as TNFa and IL-1 which may induce tissue damage.)

In contrast to its growth-stimulating effects, GM-CSF can act as a differentiation factor. Its actions on mature macrophages and neutrophils, for example, might be considered as consequences of its differentiation-inducing capacity. One way to limit the proliferation of tumour cells is to decouple

growth-factor-driven self-renewal from growth-factor-induced differentiation. In other words, the more 'differentiated' tumour cells become, the less able they are to multiply. In this regard, GM-CSF has been shown to induce differentiation of the myeloid leukaemic cell line HL60 and suppress its self-renewal. However, in several other studies, GM-CSF stimulated the proliferation of HL60 cells. Differentiation can be monitored by measuring expression of various plasma membrane-associated antigens, e.g. Leu-M3 (macrophage marker), Leu-7 (NK cell marker). These have been reported to be induced by GM-CSF in small cell lung cancer (SCLC) cell lines, suggesting that SCLC has a myeloid cell origin. This would be consistent with a proposal that SCLC arises from macrophage precursors which infiltrate damaged lung tissues, such as occur in heavy smokers. The ready availability of recombinant human GM-CSF and the limited distribution of GM-CSF receptors to cells of the myeloid and possibly erythroid lineages may thus help to define the histological origin of tumours, and suggests alternative therapeutic modalities for the treatment of cancers such as SCLC.

It thus appears that while the use of Granulocyte-macrophage colony stimulating factor (GM-CSF) has been used as a stimulant for the production of stem cells, progenitor cells, precursor cells, accessory cells and macrophages there are a substantial number of disadvantages in its use, those discussed above and the appearance of bone pain in patients to whom GM-CSF was administered, which make the use of GM-CSF not as desirable.

It is therefore an object of this invention to provide the use of another and other compounds which provide similar effects as GM-CSF but with lesser side effects.

It is a further object of this invention to provide such compounds in suitable dosages for effective and safe use.

It is still a further object of this invention to provide improved treatments and regimens of treatment.

Further and other objects of the invention will be realized by those skilled in the art from the following summary of invention and detailed description of embodiments thereof.

SUMMARY OF THE INVENTION

According to an aspect of the invention, the administration of hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) is provided for the same use as recombinant GM-CSF including the production/release of stem, progenitor and other blood cells from the bone marrow.

According to another aspect of the invention, the administration of hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate), enhance the stimulation of stem cell production/release (stem cell population) from the bone marrow.

According to another aspect of the invention, the administration of hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate), enhance the stimulation of progenitor cell production/release from the bone marrow.

According to another aspect of the invention, the administration of hyaluronic acid and pharmaceutically acceptable salts thereof (for example,

sodium hyaluronate), enhance the stimulation of precursor cell production/release from the bone marrow.

According to another aspect of the invention, the administration of hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate), enhance the stimulation of accessory cell production/release from the bone marrow.

According to another aspect of the invention, the administration of hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate), enhance the stimulation of white cell production/release from the bone marrow.

Suitable amounts of the form of hyaluronic acid comprising hyaluronic acid and pharmaceutically acceptable salts thereof may be in the order of about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered (for example, by intravenous infusion or other suitable manner) or a greater amount, such as about 6 mg/kg of patient body weight and about 12 mg/kg of patient body weight, to whom the form of hyaluronic acid is administered. Thus, suitable dosage amounts for a 70 kg. person, comprise at least about 420 mg of the form of hyaluronic acid for example, 840 mg. of the form of hyaluronic acid.

Where patients are given a regimen of treatment over a period of time for example, smaller (lesser) amounts/kg of patient body weight over a period of time (for example, every few days or once a week for a number of weeks), lesser amounts than 6 mg/kg may be used to achieve the same effect. The patient may even be "primed" to start the treatment by giving smaller/lesser dosages which,

by themselves, may not be effective. Such priming amounts may for example, be 1.5 mg/kg or 3.0 mg/kg.

The form of hyaluronic acid may be administered in any suitable carrier such as sterile water. The stimulatory effect usually commences 12 hours after administration of a form of hyaluronic acid to patients who have been primed.

One form of hyaluronic acid and/or pharmaceutically acceptable salts thereof (for example sodium salt) suitable for use with Applicant's invention is an amount having the following specifications/characteristics:

TESTS	SPECIFICATIONS	RESULTS
pH	5.0 to 7.0 at 25 degress C.	6.0
Specific Gravity	0.990 to 1.010 at 25 degress C.	1.004
Intrinsic Viscosity	4.5 to 11.0 dL/g.	7.07
Molecular Weight	178,000 to 562,000 daltons	319,378 daltons
Sodium Hyaluronate Assay and Identification	9.0 to 11.0 mg/mL. Positive	9.9 mg/ML Positive

Another such amount may comprise:

	TESTS	SPECIFICATIONS
1.	Description	White or cream odourless powder
2.	Identification (IR Spectrum)	Conforms to Ref. Std. Spectrum
3.	pH (1% solution)	5.0 to 7.0
4.	Loss on Drying	NMT 10%
5.	Residue on Ignition	15.0% to 19.0%
6.	Protein Content	NMT 0.1%
7.	Heavy Metals	NMT 20 ppm

8.	Arsenic	NMT 2 ppm
9.	Residual Solvents	
	a) Fomaldehyde	NMT 100 ppm
	b) Acetone	NMT 0.1%
	c) Ethanol	NMT 2.0%
10.	Sodium Hyaluronate Assay (dried basis)	97.0 to 102.0%
11.	Intrinsic Viscosity	10.0 to 14.5 dL/g
12.	Molecular Weight	500,000 to 800,000 daltons
13.	Total Aerobic Microbial Count (USP 23)	NMT 50 microorganisms/g
14.	Escherichia coli (USP 23)	Absent
15.	Yeasts and Moulds (USP 23)	NMT 50 microorganisms/g
16.	Bacterial Endotoxins (LAL) (USP 23)	NMT 0.07 EU/mg

Another such amount is available from Hyal Pharmaceuticals Limited and comes in a 15 ml vial of Sodium hyaluronate 20mg/ml (300mg/vial - Lot 2F3). The sodium hyaluronate amount is a 2% solution with a mean average molecular weight of about 225,000. The amount also contains water q.s. which is triple distilled and sterile in accordance with the U.S.P. for injection formulations. The vials of hyaluronic acid and/or salts thereof may be carried in a Type 1 borosilicate glass vial closed by a butyl stopper which does not react with the contents of the vial.

The amount of hyaluronic acid and/or salts thereof (for example sodium salt) may also comprise the following characteristics:

a purified, substantially pyrogen-free amount of hyaluronic acid obtained from a natural source having at least one characteristic selected from the group (and preferably all characteristics) consisting of the following:

i) a molecular weight within the range of 150,000-225,000;

ii) less than about 1.25% sulphated mucopoly-saccharides on a total weight basis;

iii) less than about 0.6% protein on a total weight basis;

iv) less than about 150 ppm iron on a total weight basis;

v) less than about 15 ppm lead on a total weight basis;

vi) less than 0.0025% glucosamine;

vii) less than 0.025% glucuronic acid;

viii) less than 0.025% N-acetylglucosamine;

ix) less than 0.0025% amino acids;

x) a UV extinction coefficient at 257 nm of less than about 0.275;

xi) a UV extinction coefficient at 280 nm of less than about 0.25; and

xii) a pH within the range of 7.3-7.9. Preferably, the hyaluronic acid is mixed with sterile water and the amount of hyaluronic acid has a mean average molecular weight within the range of 150,000-225,000 daltons. More preferably, the amount of hyaluronic acid comprises at least one characteristic selected from the group (and preferably all characteristics) consisting of the following characteristics:

i) less than about 1% sulphated mucopolysaccharides on a total weight basis;

ii) less than about 0.4% protein on a total weight basis;

iii) less than about 100 ppm iron on a total weight basis;

iv) less than about 10 ppm lead on a total weight basis;

v) less than 0.00166% glucosamine;

vi) less than 0.0166% glucuronic acid;

vii) less than 0.0166% N-acetylglucosamine;

viii) less than 0.00166% amino acids;

x) a UV extinction coefficient at 257 nm of less than about 0.23;

xi) a UV extinction coefficient at 280 nm of less than 0.19; and

xii) a pH within the range of 7.5-7.7

Applicants may also use sodium hyaluronate produced and supplied by LifeCore™ Biomedical, Inc., having the following specifications:

Characteristics	Specification
Appearance	White to cream colored particles
Odor	No perceptible odor
Viscosity Average Molecular Weight	< 750,000 Daltons
UV/Vis Scan, 190-820nm	Matches reference scan
OD, 260nm	< 0.25 OD units
Hyaluronidase Sensitivity	Positive response
IR Scan	Matches reference
pH, 10mg/g solution	6.2 - 7.8
Water	8% maximum
Protein	< 0.3 mcg/mg NaHy
Acetate	< 10.0 mcg/mg NaHy

Heavy Metals, maximum ppm

As	Cd	Cr	Co	Cu	Fe	Pb	Hg	Ni
2.0	5.0	5.0	10.0	10.0	25.0	10.0	10.0	5.0

Microbial Bioburden	None observed
Endotoxin	< 0.07EU/mg NaHy
Biological Safety Testing	Passes Rabbit Ocular Toxicity Test

Another amount of sodium hyaluronate proposed to be used is sold under the name Hyaluronan HA-M5070 by Skymart Enterprises, Inc. having the following specifications:

Specifications' Test Results	
Lot No.	HG1004
pH	6.12
Condroitin Sulfate	not detected
Protein	0.05%
Heavy Metals	Not more than 20 ppm
Arsenic	Not more than 2 ppm
Loss on Drying	2.07%
Residue on Ignition	16.69%
Intrinsic Viscosity	12.75 dl/s (XW: 679,000)
Nitrogen	3.14%
Assay	104.1%
Microbiological Counts	80/g
E. coli	Negative
Mold and Yeast	Not more than 50/g

Other forms of hyaluronic acid and/or its salts may be chosen from other suppliers and those described in prior art documents provided they are suitable.

The following references teach hyaluronic acid, sources thereof, and processes for the manufacture and recovery thereof which may be suitable.

United States Patent 4,141,973 teaches hyaluronic acid fractions (including sodium salts) having:

"(a) an average molecular weight greater than about 750,000, preferably greater than about 1,200,000 - that is, a limiting viscosity number greater than about 1400 $cm^3/g.$, and preferably greater than about 2000 $cm^3/g.$;

(b) a protein content of less than 0.5% by weight;

(c) ultraviolet light absorbance of a 1% solution of sodium hyaluronate of less than 3.0 at 257 nanometers wavelength and less than 2.0 at 280 nanometers wavelength;

(d) a kinematic viscosity of a 1% solution of sodium hyaluronate in physiological buffer greater than about 1000 centistokes, preferably greater than 10,000 centistokes;

(e) a molar optical rotation of a 0.1 - 0.2% sodium hyaluronate solution in physiological buffer of less than -11 X 10^3 degree - cm^2/mole (of disaccharide) measured at 220 nanometers;

(f) no significant cellular infiltration of the vitreous and anterior chamber, no flare in the aqueous humour, no haze or flare in the vitreous, and no pathological changes to the cornea, lens, iris, retina, and choroid of the owl monkey eye when one milliliter of a 1% solution of sodium hyaluronate dissolved in physiological buffer is implanted in the vitreous replacing approximately one-half the existing liquid vitreous, said HUA being

(g) sterile and pyrogen free and

(h) non-antigenic."

Canadian Letters Patent 1,205,031 (which refers to United States Patent 4,141,973 as prior art) refers to hyaluronic acid fractions having average

molecular weights of from 50,000 to 100,000; 250,000 to 350,000; and 500,000 to 730,000 and discusses processes of their manufacture.

Where high molecular weight hyaluronic acid (or salts) is used, it must be diluted to permit administration and ensure no coagulation or blockage.

As there is no toxicity of the form of hyaluronic acid, the form of hyaluronic acid may be administered in doses in excess of 12 mg/kg, for example, 3000 mg/70 kg person or greater without adverse toxic effects.

Thus, according to one aspect of the invention, a method of treatment is provided comprising the administration to a human of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) for enhancing (stimulating) the production/release of blood cells, even those whose increased presence in the blood may only be detected by the presence of indicator cells such as heavier, larger cells, for example, plasma cells which are indicative of the presence of stem cells.

According to another aspect of the invention, a method of treatment is provided comprising the administration to a human of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) for enhancing (stimulating) the production/release of stem cells.

According to another aspect of the invention, a method of treatment is provided comprising the administration to a human of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically

acceptable salts thereof (for example, sodium hyaluronate) for enhancing (stimulating) the production/release of progenitor cells.

According to another aspect of the invention, a method of treatment is provided comprising the administration to a human of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) for enhancing (stimulating) the production/release of precursor cells.

According to yet another aspect of the invention, a method of treatment is provided comprising the administration to a human of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) for enhancing (stimulating) the production/release of accessory cells.

According to a still yet other aspect of the invention, a method of treatment is provided comprising the administration to a human of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) for enhancing (stimulating) the production/release of white blood cells (for example, macrophages).

According to another aspect of the invention, the use of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) is provided for the manufacture of pharmaceutical composition for administration to a human for enhancing (stimulating) the production/release of stem cells.

According to another aspect of the invention, the use of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) is provided for the manufacture of pharmaceutical composition for administration to a human for enhancing (stimulating) the production/release of progenitor cells.

According to another aspect of the invention, the use of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) is provided for the manufacture of pharmaceutical composition for administration to a human for enhancing (stimulating) the production/release of precursor cells.

According to another aspect of the invention, the use of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) is provided for the manufacture of pharmaceutical composition for administration to a human for enhancing (stimulating) the production/release of accessory cells.

According to another aspect of the invention, the use of an effective amount of a form of hyaluronic acid, for example, hyaluronic acid and pharmaceutically acceptable salts thereof (for example, sodium hyaluronate) is provided for the manufacture of pharmaceutical composition for administration to a human for enhancing (stimulating) the production/release of white blood cells (for example, macrophages).

According to yet another aspect of the invention, the use of hyaluronic acid and pharmaceutically acceptable salts thereof is provided for stimulating the production/release of stem cells.

According to yet another aspect of the invention, the use of hyaluronic acid and pharmaceutically acceptable salts thereof is provided for stimulating the production/release of progenitor cells.

According to yet another aspect of the invention, the use of hyaluronic acid and pharmaceutically acceptable salts thereof is provided for stimulating the production/release of precursor cells.

According to yet another aspect of the invention, the use of hyaluronic acid and pharmaceutically acceptable salts thereof is provided for stimulating the production/release of accessory cells.

According to yet another aspect of the invention, the use of hyaluronic acid and pharmaceutically acceptable salts thereof is provided for stimulating the production/release of white blood cells (for example, macrophages).

Thus, by administering effective amounts of the forms of hyaluronic acid, patients can be treated with the form of hyaluronic acid which is safe and non-toxic and the patient does not suffer the adverse side effects of recombinant GM-CSF treatment, yet achieves results that are achieved by the administration of recombinant GM-CSF. By administering a regimen of treatment comprising a plurality of dosages of hyaluronic acid over a period of time (for example, several weeks) or dosages comprising an amount or amounts which is/are lesser amount(s) than (an) effective amount(s) followed by amounts which are suitable, effective amounts, the patient is first "primed" and the subsequent

administration achieves the desired results in the patient. Lesser amounts than the amounts used without priming may even be required to be effective in the patient to stimulate the production/release of the cells when the patient is primed. For example, suitable dosage amounts may be 6 mg/kg of patient body weight or 12 mg of the form of hyaluronic acid/kg patient body weight. A suitable regimen may also comprise a less than suitable amount (for example 1.5 mg of the form of hyaluronic acid/kg patient body weight or 3.0 mg/kg for "priming" purposes followed by administration of a suitable amount (for example, about 6 mg/kg, 10 mg/kg or more after a pre-determined interval or intervals. Another suitable regimen of sustained treatment may be provided as follows:

Week 1: 1.5 mg/kg (primer);
Week 2: (7 days later) - 3.0 mg/kg (primer);
Week3: (7 days later) - 6 mg/kg (suitable amount);
Week 4: (7 days later) - 12 mg/kg (suitable amount);

The treatment at Week 3 or 4 may be continued in Weeks 5, 6, 7, etc. for as long as required. Any of the treatments may be continued for as long as required.

Thus, for loss of blood, leukemias, anaemic individuals, blood transfusions, it will be useful to stimulate white cell production/release and/or production of other cells in the patient.

Embodiments of the invention will now be illustrated with reference to the following Figures in which:

Figues 1-6 depict the results of the technique of cell sorting that sorts cells, in this case, the white cells (the red cells are too small and are not included in the

cells as sorted) according to their cell surface characteristics (using known antibodies to detect them) and their sizes, taken from healthy individuals who were administered the form of hyaluronic acid, sodium hyaluronate, at time "0" and from whom blood was drawn at time: 0 (Figure 1); 1 hour after administration of sodium hyaluronate (Figure 2); 4 hours after administration (Figure 3); 12 hours after administration (Figure 4); 24 hours after administration of sodium hyaluronate (Figure 5); and 72 hours after administration (Figure 6).

Thus, Figure 1 (and the other Figures 2-6) provide plots of cell characteristics including granularity (plotted vertically) and forward scatter (FSC-height) relating to size of cells (plotted horizontally). The Plot shows different fractions labelled G_1 to G_4 (corresponding to R_1 to R_4 with R_5 being the entire field) of which G_4 is the one of concern displaying changes in plasma cells (being big cells) which have been pushed out of the bone marrow. (R_5 is the entire field of sorted cells.) The presence of the plasma cells which are indicators of the increase production/release of stem cells, are thus indicative of the presence of stem cells (which are small and therefore are minimally scattered and are not specifically sorted or directly detected in this assay) and precursor cells which are pushed out (released) from the bone marrow.

Because the group identified as G_4 are the large cells (for example, plasma cells), their "Granularity" and "forward scatter" push them when being sorted by the techniques known to persons skilled in the art up and away from the vertex.

It is therefore clear that the percentage of the cells in G_4 relative to all the cells in the field. (R_5) which comprises G_1 (which corresponds to R_1), G_2 (which corresponds to R_2), G_3 (which corresponds to R_3), and G_4 which corresponds to R_4) and the others found in R_5 not previously accounted for are with respect to:

Figure 1 (after 0 hours) → 5% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

Figure 2 (after 1 hour) → 9% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

Figure 3 (after 4 hours) → 26% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

Figure 4 (after 12 hours) → 30% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

Figure 5 (after 24 hours) → 9% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

Figure 6 (after 72 hours) → 6% calculated as $\frac{(G_4)}{(R5)} \times 100\%$

(Each of Figures 1-6 is accompanied by supporting data and chart plotting. Counts v. FSC-Height)

The data shown is based on examples wherein the amount of sodium hyaluronate equals or exceeds 6 mg/kg of body weight per patient (i.e. 6 mg/kg and 12 mg/kg) which provide very similar results. Before administering the 6 mg/kg and 12 mg/kg amounts, patients were administered 1.5 mg/kg and 3.0 mg/kg as discussed.

The characteristics of the sodium hyaluronate used with the protocols are set out below:

"A"

TESTS	SPECIFICATIONS	RESULTS
pH	5.0 to 7.0 at 25 degress C.	6.0
Specific Gravity	0.990 to 1.010 at 25 degress C.	1.004
Intrinsic Viscosity	4.5 to 11.0 dL/g.	7.07
Molecular Weight	178,000 to 562,000 daltons	319,378 daltons
Sodium Hyaluronate Assay and Identification	9.0 to 11.0 mg/mL. Positive	9.9 mg/ML Positive

Another amount may comprise:

	TESTS	SPECIFICATIONS
1.	Description	White or cream odourless powder
2.	Identification (IR Spectrum)	Conforms to Ref. Std. Spectrum
3.	pH (1% solution)	5.0 to 7.0
4.	Loss on Drying	NMT 10%
5.	Residue on Ignition	15.0% to 19.0%
6.	Protein Content	NMT 0.1%
7.	Heavy Metals	NMT 20 ppm
8.	Arsenic	NMT 2 ppm
9.	Residual Solvents	
	a) Fomaldehyde	NMT 100 ppm
	b) Acetone	NMT 0.1%
	c) Ethanol	NMT 2.0%
10.	Sodium Hyaluronate Assay (dried basis)	97.0 to 102.0%
11.	Intrinsic Viscosity	10.0 to 14.5 dL/g
12.	Molecular Weight	500,000 to 800,000 daltons
13.	Total Aerobic Microbial Count (USP 23)	NMT 50 microorganisms/g
14.	Escherichia coli (USP 23)	Absent

15.	Yeasts and Moulds (USP 23)	NMT 50 microorganisms/g
16.	Bacterial Endotoxins (LAL) (USP 23)	NMT 0.07 EU/mg

The following protocol was followed for administering the sodium hyaluronate identified at page 20, line 33 to page 21, line 5 and the drawing of blood from the patients to whom the sodium hyaluronate was administered.

Four healthy non-smoking female volunteers and four healthy non-smoking male volunteers were given, at different times (at least 7 days between dosages) the following dosages:

(A) 1.5 mg/kg body weight, intravenous infusion of sterile 1% hyaluronic acid solution.

(B) 3.0 mg/kg body weight, intravenous infusion of sterile 1% hyaluronic acid solution.

(C) 6.0 mg/kg body weight, intravenous infusion of sterile 1% hyaluronic acid solution.

(D) 12.0 mg/kg body weight, intravenous infusion of sterile 1% hyaluronic acid solution.

(The hyaluronic acid solution was as described herein as "A" at page 22 of this document.)

In each case, a total volume of 250 ml was infused. Therefore, the 1% hyaluronic acid solution as required was diluted with an appropriate volume of 0.9% sodium chloride solution. The infusion was over a period of 120 minutes.

Each of the dosages was administered in an ascending manner (1.5 mg/kg, 3.0 mg/kg, 6.0 mg/kg, and 12.0 mg/kg) to each of the individuals with at least 7 days between doses. The individuals were asked to engage in normal activity for the first four hours after drug administration avoiding both vigorous exertion and complete rest.

Blood samples were drawn from each person at time intervals of 0, 1, 4, 12, 24 and 72 hours after the administration of each of the dosages.

The cells in the drawn samples were sorted by known cell sorting techniques. Figures 1-6 illustrate the results of sorting the white cells in the blood samples taken from the individuals after 0, 1, 4, 12, 24, and 72 hours after administration of 12 mg/kg of body weight of the sodium hyaluronate by intravenous infusion of the individuals. (Red cells are, because of their small size, not shown in the data.)

Area G_4 displays large cells in the blood samples. These cells include plasma cells, polymorphonuclear cells (such as granulocytes, neutrophils, and the like). Normally, at time, t = 0 hours [at infusion], only small amounts of these cells are present in normal blood (5% of the total of white cell [R_5] population. After administration of 12 mg of sodium hyaluronate/kg of body weight, their presence increases.

time (t) hours	% of Cell Population
1	9.47
4	25.88
12	30.03
24	9.10
72	6.80

These large cells are released from the bone marrow and are indicators of the presence stem cells.

Because the plasma cells increase in the samples as the presence of stem cells increase, the increase in the plasma cells indicates that the stem cells present have also increased. The stem cells present in the sample increase because they have been released from (pushed out from) the bone marrow. Thus, the administration of sodium hyaluronate (M.W. 319,378 daltons intrinsic viscosity 7.07 dL/g.) in an amount of 12 mg/kg to an individual causes the stem cell production to be stimulated in the individual (increased stem cell production). If stem cells are stimulated to be produced (more than usual), the stimulation of progenitor cell production, precursor cell production, accessory cell production, and macrophage production among other cells whose production is also stimulated, also occurs thereby increasing the presence of these cells in the body to benefit the individual to whom the sodium hyaluronate was administered. The result is the mobilization of more cells with which to fight disease.

Thus, instead of using recombinant GM-CSF with its adverse effects, the individual may now receive a form of hyaluronic acid (without the same side effects). Because of a lack of toxicity, greater amounts than 12 mg/kg of body weight may be administered to a patient for the effect. I have also found that an

amount of 6 mg sodium hyaluronate/kg of body weight administered to an individual has the similar effect as the administration of 12 mg sodium hyaluronate/kg of body weight. Lesser amounts than 6 mg/kg may be admiistered to achieve similar effects. However, the administration of an amount of 3 mg of sodium hyaluronate/kg of body weight had little, if any, effect and the administration of an amount of 1 mg of sodium hyaluronate/kg of body weight had no effect. However, the 1 mg/kg and 3 mg/kg of patient weight may "prime" the patient so that lesser amounts of the form of hyaluronic acid may be suitable to be effective to stimulate the production/release of the blood cells than 6 mg/kg.

Thus, for example, cancer patients who are administered recombinant GM-CSF to mobilize stem and other cell production, may now be administered effective amounts of sodium hyaluronate to mobilize stem and other cells for example, polymorphs (such as non-specific scavengers for example, phagocytes). Other patients suffering a disease whose treatment would benefit from increasing production (stimulating production) and release of these cells, may also be administered effective amounts of sodium hyaluronate. Additionally, the administration may continue with small amounts at first, increasing over a prolonged period to first "prime" the patient to further treatment using "effective" forms of hyaluronic acid. The continuous administration of suitable dosage amounts at predetermined intervals (for example, weekly intervals) may also be used to provide a sustained production/release of blood cells over a prolonged period of time.

Thus, forms of hyaluronic acid and pharmaceutically acceptable salts thereof such as sodium hyaluronate may be used instead of recombinant GM-CSF for purposes known for using GM-CSF without the same side effects including accompanying bone pain. Additionally, while sodium hyaluronate

need only be administered once to achieve the same effects that are achieved by using GM-CSF which is normally administered by several injections over 48 hours, the Sodium hyaluronate may also be administered continuously at varying dosages to treat a patient to provide a sustained production/release of blood cells.

Combinations of any of the above may also be used to benefit the patient.

As many changes can be made to the embodiments without departing from the scope of the invention, it is intended that all material contained herein be interpreted as illustrative of the invention and not in a limiting sense.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE AS FOLLOWS:

1. The use of forms of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof for the same purposes known for using recombinant GM-CSF.

2. A method of treating a patient for the same purposes as recombinant GM-CSF is used, the method comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof to the patient.

3. The use of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof in the manufacture of a pharmaceutical composition for administration to a human for the same purposes as recombinant GM-CSF is administered.

4. The use of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof for enhancing the stimulation of stem cell production, (stem cell population) and thus, progenitor cell production, precursor cell production, accessory cell production and macrophage production in a human.

5. A method of treating a patient for enhancing the stimulation of stem cell production, (stem cell population) and thus, progenitor cell production, precursor cell production, accessory cell production and macrophage production in a human, comprising administering an effective amount of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof to the patient.

6. The use of a form of hyaluronic acid selected from the group consisting of hyaluronic acid and pharmaceutically acceptable salts thereof in the manufacture of a pharmaceutical composition for administration to a human for the same purposes as recombinant GM-CSF is administered for enhancing the stimulation of stem cell production, (stem cell population) and thus, progenitor cell production, precursor cell production, accessory cell production and macrophage production in a human.

7. The use of Claim 1, 3, 4, or 6 wherein the form of hyaluronic acid comprising hyaluronic acid and pharmaceutically acceptable salts thereof is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

8. The method of Claim 2 or 5 wherein the form of hyaluronic acid comprising hyaluronic acid and pharmaceutically acceptable salts thereof is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

9. The use of Claim 7 wherein the form of hyaluronic acid is at least about 12 mg/kg of patient body weight.

10. The method of Claim 8 wherein the form of hyaluronic acid is at least about 12 mg/kg of patient body weight.

11. The use of Claim 1, 3, 4, 6, 7, or 9 wherein the form of hyaluronic acid has a molecular weight less than about 750,000 daltons.

12. The use of Claim 11 wherein the form of hyaluronic acid is sodium hyaluronate.

13. The method of Claim 2, 5, 8, or 10 wherein the form of hyaluronic acid has a molecular weight less than about 750,000 daltons.

14. The method of Claim 13 wherein the form of hyaluronic acid is sodium hyaluronate.

15. The use of Claim 11 or 12 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

16. The method of Claim 13 or 14 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

17. A process for the administration to a human of an effective amount of a form of hyaluronic acid comprising administering to the human an effective amount of a form of hyaluronic acid selected from the group of hyaluronic acid and pharmaceutically acceptable salts thereof for enhancing (stimulating) the production of stem cells.

18. A process for the administration to a human of an effective amount of a form of hyaluronic acid comprising administering to the human an effective amount of a form of hyaluronic acid selected from the group of hyaluronic acid and pharmaceutically acceptable salts thereof for enhancing (stimulating) the production of progenitor cells.

19. A process for the administration to a human of an effective amount of a form of hyaluronic acid comprising administering to the human an effective

amount of a form of hyaluronic acid selected from the group of hyaluronic acid and pharmaceutically acceptable salts thereof for enhancing (stimulating) the production of precursor cells.

20. A process for the administration to a human of an effective amount of a form of hyaluronic acid comprising administering to the human an effective amount of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof for enhancing (stimulating) the production of accessory cells.

21. A process for the administration to a human of an effective amount of a form of hyaluronic acid comprising administering to the human an effective amount of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof for enhancing (stimulating) the production of macrophages.

22. The process of Claim 17, 18, 19, 20, or 21 wherein the form of hyaluronic acid has a molecular weight less than about 750,000 daltons.

23. The process of Claim 17 or 22 wherein the form of hyaluronic acid is sodium hyaluronate.

24. The process of Claim 22 or 23 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

25. The process of Claim 17, 22, 23, or 24 wherein the form of hyaluronic acid comprising hyaluronic acid and pharmaceutically acceptable salts thereof is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

26. The use of an effective amount of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof for the manufacture of pharmaceutical composition for administration to a human for enhancing (stimulating) the production of stem cells.

27. The use of an effective amount of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof for the manufacture of pharmaceutical composition for administration to a human for enhancing (stimulating) the production of progenitor cells.

28. The use of an effective amount of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof for the manufacture of pharmaceutical composition for administration to a human for enhancing (stimulating) the production of precursor cells.

29. The use of an effective amount of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof for the manufacture of pharmaceutical composition for administration to a human for enhancing (stimulating) the production of accessory cells.

30. The use of an effective amount of a form of hyaluronic acid selected from hyaluronic acid and pharmaceutically acceptable salts thereof for the manufacture of pharmaceutical composition for administration to a human for enhancing (stimulating) the production of macrophages.

31. The use of Claim 26, 27, 28, 29, or 30 wherein the form of hyaluronic acid has a molecular weight less than about 750,000 daltons.

32. The use of Claim 26 or 31 wherein the form of hyaluronic acid is sodium hyaluronate.

33. The use of Claim 31 or 32 wherein the form of hyaluronic acid has a molecular weight of about 320,000 daltons.

34. The use of Claim 26, 31, 32, or 33 wherein the form of hyaluronic acid comprising hyaluronic acid and pharmaceutically acceptable salts thereof is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.

35. The use of hyaluronic acid and pharmaceutically acceptable salts thereof for stimulating the production of stem cells.

36. The use of hyaluronic acid and pharmaceutically acceptable salts thereof for stimulating the production of progenitor cells.

37. The use of hyaluronic acid and pharmaceutically acceptable salts thereof for stimulating the production of precursor cells.

38. The use of hyaluronic acid and pharmaceutically acceptable salts thereof for stimulating the production of accessory cells.

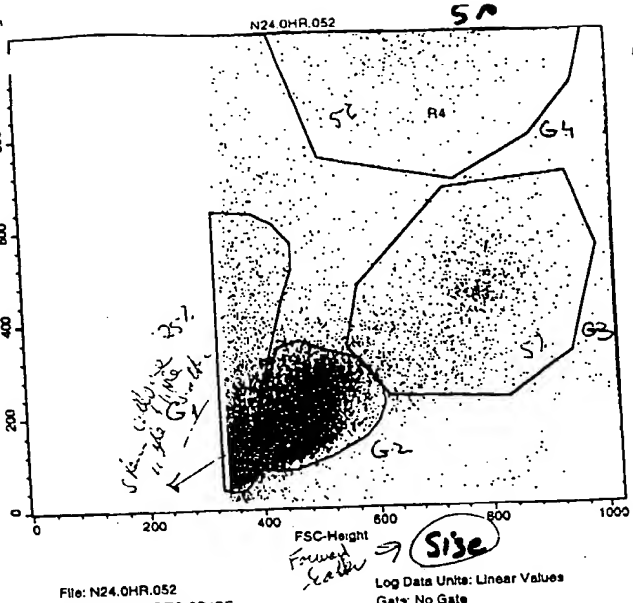
39. The use of hyaluronic acid and pharmaceutically acceptable salts thereof for stimulating the production of macrophages.

40. The use of Claim 35, 36, 37, 38, or 39 wherein the form of hyaluronic acid has a molecular weight less than about 750,000 daltons.

41. The use of Claim 35 or 40 wherein the form of hyaluronic acid is sodium hyaluronate.

42. The use of Claim 40 or 41 wherein the form of hyaiuronic acid ha- a molecular weight of about 320,000 daltons.

43. The use of Claim 35, 40, 41, or 42 wherein the form of hyaluronic acid comprising hyaluronic acid and pharmaceutically acceptable salts thereof is at least about 6 mg/kg of patient body weight to whom the form of hyaluronic acid is administered.



File: N24.0HR.052
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Gated Events: 20235
X Parameter: FSC-H FSC-Height (Linear)

Log Data Units: Linear Values
Gate: No Gate
Total Events: 20235
Y Parameter: SSC-H SSC-H (Linear)

Region	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	5091	25.16	25.16	362.40	362.02	143.68	122.43	1, 2
R2	11752	58.08	58.08	460.67	458.78	183.54	174.82	1, 2
R3	1046	5.17	5.17	733.07	727.10	415.72	403.53	1, 2
R4	957	4.73	4.73	703.43	697.21	1002.78	1001.41	1, 2
R5	19853	98.11	98.11	461.99	451.20	230.98	182.21	1, 0

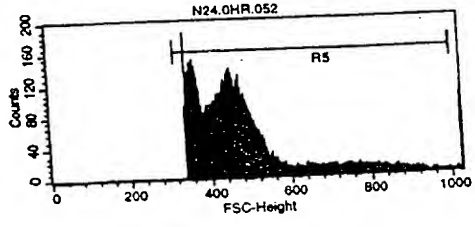

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FIGURE 1

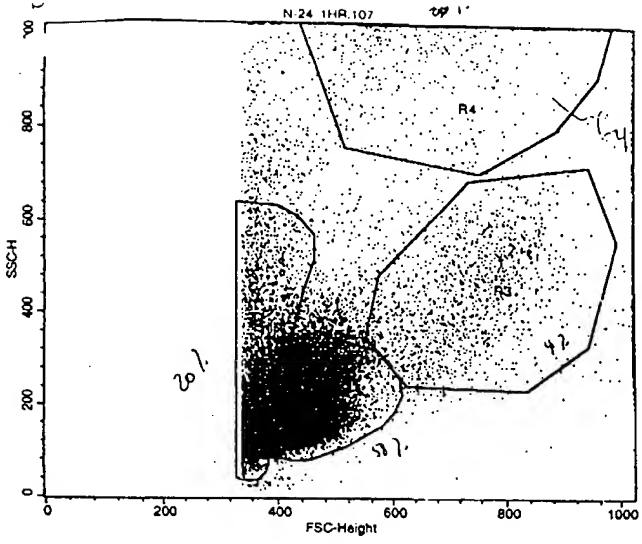

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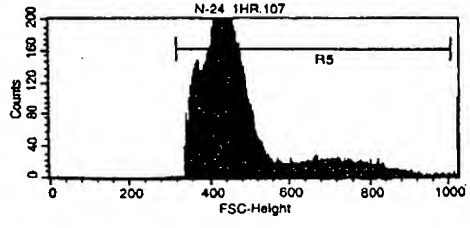
FIGURE 2

File: N-24 1HR.107
Sample ID: HAFITC CD4PE
Gated Events: 29655
X Parameter: FSC-H FSC-Height (Linear)

Log Data Units: Linear Values
Gate: No Gate
Total Events: 29655
Y Parameter: SSC-H SSC-H (Linear)

Region	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	5929	19.99	19.99	371.93	371.42	229.53	198.58	1, 2
R2	17181	57.94	57.94	449.78	448.35	194.16	184.07	1, 2
R3	1316	4.44	4.44	721.78	716.46	435.30	422.11	1, 2
R4	2809	9.47	9.47	694.70	687.82	1009.13	1007.87	1, 2
R5	29255	98.65	98.65	470.17	459.19	304.03	236.98	1, 0



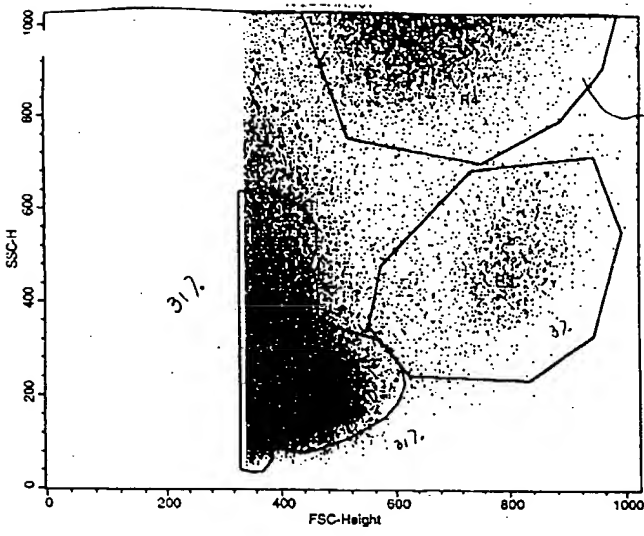
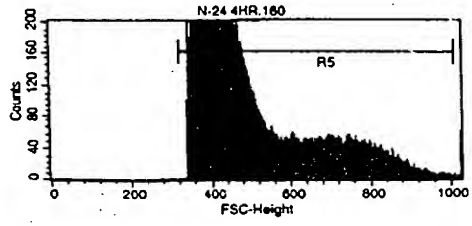


FIGURE 3

File: N-24 4HR.161
Sample ID: HAFITC CD4PE
Gated Events: 50000
X Parameter: FSC-H FSC-Height (Linear)
Log Data Units: Linear Values
Gate: No Gate
Total Events: 50000
Y Parameter: SSC-H SSC-H (Linear)

Region	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	15374	30.75	30.75	374.55	373.82	302.56	271.09	1, 2
R2	15359	30.72	30.72	452.13	450.52	200.91	192.99	1, 2
R3	1509	3.02	3.02	764.50	759.01	477.03	465.53	1, 2
R4	12940	25.88	25.88	688.23	679.12	997.82	996.43	1, 2
R5	49787	99.57	99.57	498.00	479.12	481.11	367.27	1, 0



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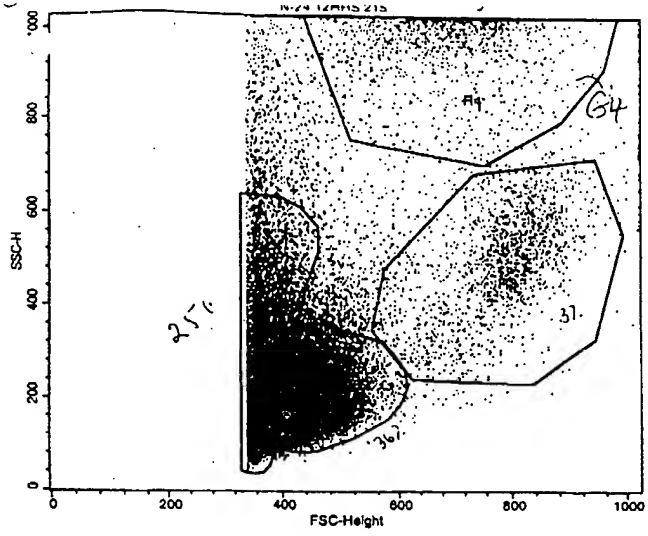
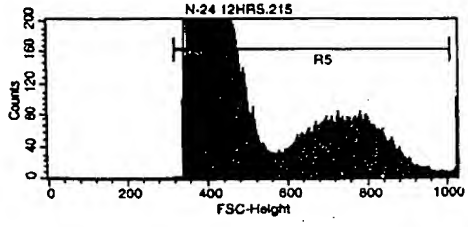


FIGURE 4

File: N-24 12HRS.215
Sample ID: HAFITC CD4PE
Gated Events: 50000
X Parameter: FSC-H FSC-Height (Linear)

Log Data Units: Linear Values
Gate: No Gate
Total Events: 50000
Y Parameter: SSC-H SSC-H (Linear)

Region	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	11868	23.74	23.74	369.87	369.33	244.22	211.18	1, 2
R2	18416	36.83	36.83	448.65	447.39	184.25	177.16	1, 2
R3	1631	3.26	3.26	770.24	765.15	485.71	472.28	1, 2
R4	15013	30.03	30.03	727.15	720.73	1017.53	1017.07	1, 2
R5	49637	99.27	99.27	524.97	502.11	476.63	337.95	1, 0



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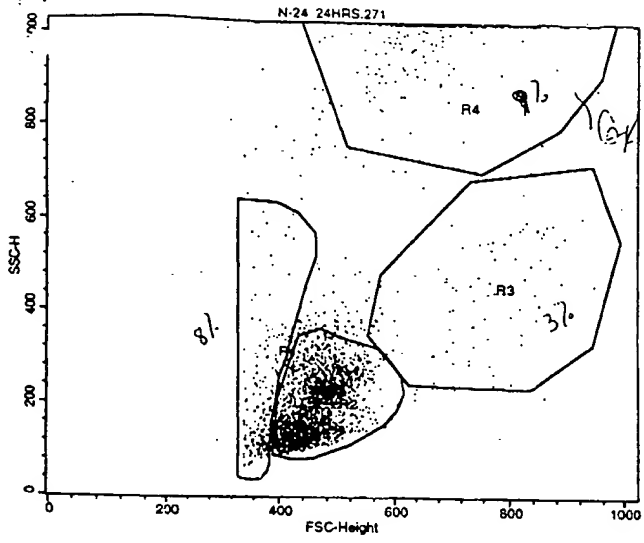
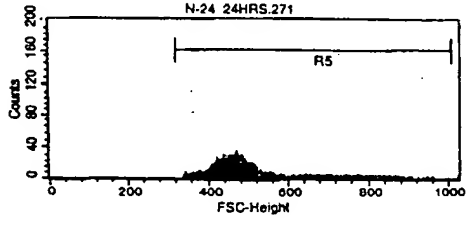


FIGURE 5

File: N-24 24HRS.271
Sample ID: HAFITC CD4PE
Gated Events: 3000
X Parameter: FSC-H FSC-Height (Linear)

Log Data Units: Linear Values
Gate: No Gate
Total Events: 3000
Y Parameter: SSC-H SSC-H (Linear)

Region	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	241	8.03	8.03	372.93	372.39	200.17	172.29	1, 2
R2	2227	74.23	74.23	463.11	461.59	190.64	180.25	1, 2
R3	91	3.03	3.03	736.37	729.14	428.80	415.01	1, 2
R4	273	9.10	9.10	675.01	668.46	997.55	996.15	1, 2
R5	2993	99.77	99.77	484.34	476.32	284.63	223.45	1, 0



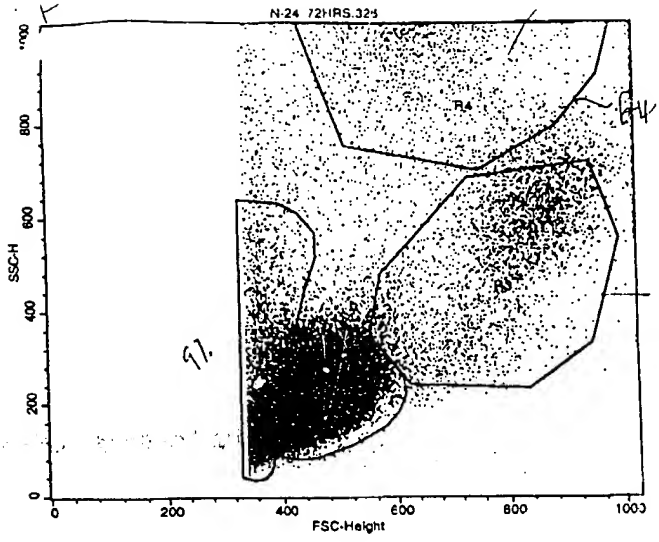
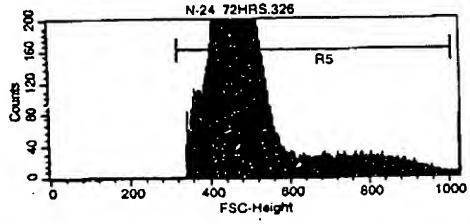


FIGURE 6

File: N-24 72HRS.326	Log Data Units: Linear Values
Sample ID: HAFITC CD4PE	Gate: No Gate
Gated Events: 50000	Total Events: 50000
X Parameter: FSC-H FSC-Height (Linear)	Y Parameter: SSC-H SSC-H (Linear)

Region	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	4631	9.26	9.26	371.76	371.26	219.42	192.19	1, 2
R2	36587	73.17	73.17	466.61	467.14	205.82	190.29	1, 2
R3	2911	5.82	5.82	776.01	768.40	501.23	482.26	1, 2
R4	3399	6.80	6.80	694.12	687.24	982.82	979.60	1, 2
R5	49829	99.66	99.66	496.29	485.45	289.31	240.51	1, 0



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